Convergent adaptation to a marginal habitat by homoploid hybrids and polyploid ecads in the seaweed genus *Fucus*

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Hybridization and polyploidy are two major sources of genetic variability that can lead to adaptation in new habitats. Most species of the brown algal genus *Fucus* are found along wave-swept rocky shores of the Northern Hemisphere, but some species have adapted to brackish and salt marsh habitats. Using five microsatellite loci and mtDNA RFLP, we characterize two populations of morphologically similar, muscoides-like *Fucus* inhabiting salt marshes in Iceland and Ireland. The Icelandic genotypes were consistent with *Fucus vesiculosus*×*Fucus spiralis* F₁ hybrids with asymmetrical hybridization, whereas the Irish ones consisted primarily of polyploid *F. vesiculosus*.

1. INTRODUCTION

Natural hybridization has long been recognized and studied for its role in the evolution of plant and animal species (Arnold 1997; Mallet 2005). As an important mechanism of speciation, especially in plants, the resulting novel genetic variation will be subjected to natural selection from divergent ecological pressures (see Gross *et al.* 2004 and references therein). In some cases, such as irises and sunflowers, hybrids have a fitness advantage in habitats substantially different from that of either parental species (Cruzan & Arnold 1993; Rieseberg *et al.* 2003). Another widely recognized mechanism of speciation is polyploidy, which can be a major source of increased genetic diversity and concomitant adaptability (Wendel 2000). We report here that in the seaweed genus *Fucus*, both homoploid hybrids and a polyploid non-hybrid derived from one of the parental species have convergent morphologies and have successfully adapted to a salt marsh habitat differing considerably from the intertidal pool and rocky coast typically inhabited by the parents.

Fucus species are dominant members of the intertidal and shallow subtidal communities along North Atlantic and North Pacific coasts (Lüning 1990). Although most species inhabit rocky and wave-swept shores, a few are commonly found in well-drained muddy/sandy areas at the uppermost extent of tidal influence in salt marshes. Perhaps because of the reduced water motion, some salt marsh species do not have holdfasts and are termed 'ecads' (habitat-determined morphology; see Wallace *et al.* 2004). Ecads can be entangled amongst salt marsh vascular plants or embedded in mud/sand; both forms typically form thick mats that often extend beyond the high intertidal zone. In addition to lacking a holdfast, the salt marsh ecads are distinguished from the attached species (from which they are derived) by a dwarf morphology and sterility, propagating only by vegetative reproduction (see references in Wallace *et al.* 2004).

The *Fucus* ecad found in European (UK and continental) salt marshes is identified as *Fucus cottonii*, whereas in Maine (USA) it is termed a 'muscoides-like *Fucus*' (m-l*F*; reviewed in Wallace *et al.* (2004)). Recently, Wallace *et al.* (2004) indicated that populations of m-l*F* in Maine consisted primarily of F₁ hybrids between *Fucus vesiculosus* (*Fv*) and *Fucus spiralis* (*Fsp*).

The presence of m-l*F* in Iceland (previously unreported), as well as on both sides of the North Atlantic (Maine, Ireland), provides an unique opportunity to investigate the nature and generality of hybridization and adaptation to salt marshes by *Fucus*. Accordingly, we examined the microsatellite genotypes of m-l*F* from salt marshes of Iceland and Ireland and used mtDNA to determine directionality of hybridization.

2. MATERIAL AND METHODS

(a) *Sampling and DNA extraction*
Samples of all species were collected in salt marshes on the southern shore of Osar, near Hafnir, Iceland (m-l*F*=5, *Fsp*=17, *Fv*=14; every individual separated by greater than or equal to 1 m) and near Ros Muc (*Fv*=15, m-l*F*=19) and 30 km distant in Spiddal (*Fsp*=15), County Galway, Ireland (see appendix I of the electronic supplementary material). Tissue was stored on silica crystals and DNA extracted and purified as described earlier (Coyer *et al.* 2002c).

(b) *Microsatellite genotyping*
Five microsatellite loci were genotyped using protocols for loci L20, L38, L58, L94 (Engel *et al.* 2003) and B3 (C. Perrin 2006, unpublished data). The loci differed from the four used by Wallace *et al.* (2004), as their loci could not be consistently amplified in all samples from both Iceland and Ireland (data not shown). Genotypes were visualized on an ABI 377 autosequencer and GENESCAN software (Applied Biosystems).

(c) *mtDNA RFLP*
We sequenced the mtDNA spacer region (Coyer *et al.* 2006) from 27 individuals: *Fv* (8 Ireland, 1 Iceland), *Fsp* (8 Ireland, 4 Iceland), and m-l*F* (4 Ireland, 2 Iceland). The aligned sequences revealed a single position (A/T) that separated *Fv* and *Fsp*, which fortuitously was located in an *xba*I restriction site. Thus, RFLP analysis was used for all samples to distinguish the mtDNA of *Fsp* (one fragment of 600–700 bp) from *Fv* (two fragments of *ca* 350 bp). Digestion was according to the manufacturers protocol (Promega).

(d) *Data analysis*
Microsatellite genotypes were analysed with STRUCTURE (Pritchard *et al.* 2000), which uses a Bayesian algorithm to identify *K* user-defined clusters of individuals that are genetically homogeneous. Sampled individuals are assigned either to clusters or jointly to two or more clusters if their genotypes indicate admixture. This approach has been used to study hybridization in *Fucus* (Engel *et al.* 2005), and as it is based on probability, it is more powerful than the graphical approaches, such as factorial correspondence analysis, that have been used previously (Coyer *et al.* 2002a;

Wallace *et al.* 2004). Samples from Iceland and Ireland were analysed independently. All analyses were replicated 10 times to ensure proper convergence of the MCMC with the parameters: ancestry model=admixture (to account for recent divergence and shared ancestral polymorphisms); frequency model=independent; burn-in=50 000; MCMC length=2 000 000 after burn-in. To test whether m-lF were hybrids, we first ran STRUCTURE with $K=2$ and if hybrids were not apparent, a second analysis was performed with $K=3$.

3. RESULTS AND DISCUSSION

(a) Iceland

The m-lF has not been reported from Iceland (Gunnarsson & Jónsson 2002), perhaps because of its highly localized occurrence along the perimeter of discrete pools (see appendix I of the electronic supplementary material). Microsatellite analyses were consistent with all muscoides-like individuals sampled in Iceland being F_1 hybrids between *Fsp* and *Fv* (figure 1a), a pattern identical to that found in a Maine (USA) estuary by Wallace *et al.* (2004). Furthermore, mtDNA RFLP analysis revealed that the Icelandic hybrids had mtDNA characteristic of *Fsp*. Since mtDNA is maternally inherited in *Fucus* (Coyer *et al.* 2002b), hybridization appeared to be asymmetrical with all individuals stemming from *Fsp* eggs and *Fv* sperm (figure 1a). Asymmetrical hybridization has also been reported between sister taxa *Fucus serratus* and *Fucus evanescens* (Coyer *et al.* 2002a). Significantly, the mother species (*Fsp*, *F. evanescens*) in both hybridization events is hermaphroditic and the father (*Fv*, *F. serratus*) dioecious. Selfing is common in both hermaphroditic species (Coleman & Brawley 2005; Engel *et al.* 2005; J. A. Coyer & G. Hoarau 2004, unpublished data). The apparent generality of asymmetrical hybridization in *Fucus* involving a hermaphroditic mother and a dioecious father may be due to differences in sperm–egg recognition proteins and/or to the production of substantially fewer sperm per egg in hermaphroditic species of *Fucus* (40 : 1) relative to dioecious species (400 : 1) (Vernet & Harper 1980; Billard *et al.* 2005).

How did the hybrids arrive and how are they maintained in a high intertidal salt marsh, given a lower intertidal or pool habitat of the parental species? Tidal drift of fragments with receptacles from the nearest attached populations occurs and is important (J. A. Coyer 2004, personal observation), either as an ongoing source–sink process or as a single colonization event maintained by clonal propagation as in Northern Baltic *Fv* (Tatarenkov *et al.* 2005).

(b) Ireland

In sharp contrast, m-lF in Ireland were not F_1 hybrids, but *Fv*, an observation also supported by the mtDNA RFLP (figure 1b). Expanded analysis with $K=3$ (figure 1c), however, revealed that the m-lF were genetically distinct from typical *Fv*. Microsatellite traces repeatedly revealed three peaks in two loci (L20, B3) in 68% of the Irish m-lF, consistent with at least partial genome duplication and strongly suggesting polyploidy (figure 2). Thus, m-lF individuals, which are identical in morphology and ecological habitat, may be of hybrid or polyploid origin.
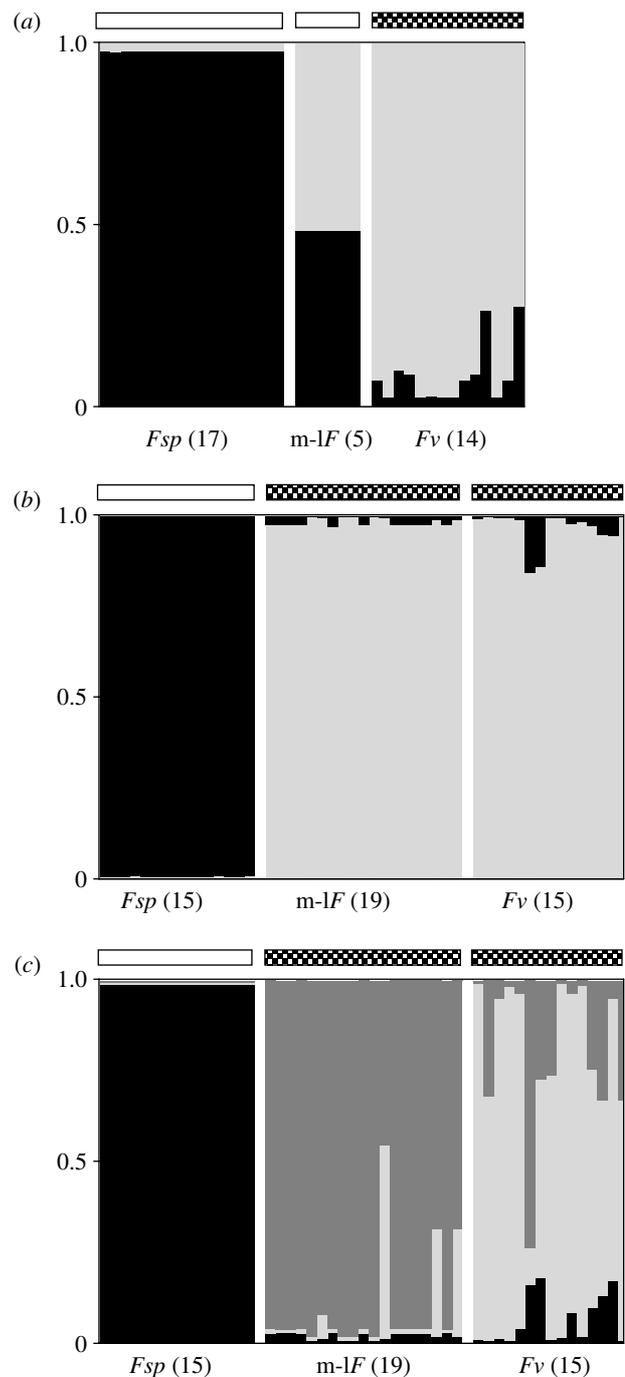
Figure 1. mtDNA characterization and detection of hybrids by STRUCTURE. The top portion of each panel indicates type of mtDNA as determined by RFLP: white, *F. spiralis*; stippled, *F. vesiculosus*. The lower portion of each panel is a plot in which each individual is represented by a vertical bar partitioned into shaded segments with a length proportional to the individual's membership in the K clusters. Each colour (black, dark grey, light grey) represents a cluster. (a) Iceland, $K=2$, ln likelihood=−234.1; (b) Ireland, $K=2$, ln likelihood=−526.2; (c) Ireland, $K=3$, ln likelihood=−457.7. *Fucus spiralis* (*Fsp*), *F. vesiculosus* (*Fv*), muscoides-like *Fucus* (m-lF); sample numbers in parentheses.

The similarities between F_1 hybrids and the polyploids suggest that *Fv* provides genes to the hybrid genome that are crucial for salt marsh existence. Substantial intraspecific variation is common in *Fv* (Powell 1963) and is a necessary prerequisite for local adaptation to environments like the brackish
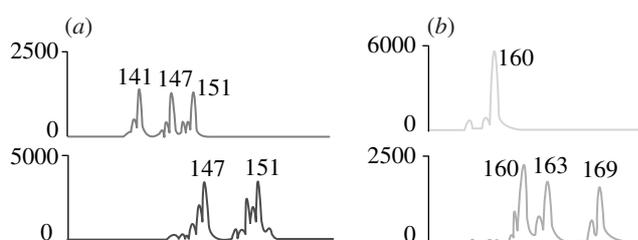
Figure 2. Microsatellite traces for loci (*a*) B3 and (*b*) L20. Duplication events could not be detected in the remaining three loci as each was characterized by only two alleles. The multiple-peak pattern was repeatable with multiple PCR reactions and consistent with at least partial genome duplication, strongly suggesting polyploidy. Both individuals revealing polyploidy at locus B3 possessed alleles 141, 147, 151, whereas nine of the 11 polyploid individuals for locus L20 had allele sizes of 160, 163, 169, one with 155, 163, 224 and another with 160, 167, 224. All alleles for both loci were common in the non-polyploid individuals; therefore spurious amplification of other genome regions was unlikely. Multiple peaks could be a result of chimaeras, but such fusion has never been demonstrated in *Fucus* despite repeated attempts. In contrast, none of the *Fv* individuals showed more than two alleles for these loci.

(3–7 psu) Baltic Sea, where it is widely distributed and the only perennial fucoid (Kautsky & Kautsky 2000). Moreover, Baltic Sea populations of *Fv* are permanently submerged in brackish water and, consequently, more tolerant of low salinity (Serrão *et al.* 1996) and less tolerant of emersion stress (Pearson *et al.* 2000). Recent genetic analysis also has revealed a common origin between *Fv* and the newly described *Fucus radicans* in the northern Baltic Sea (Bergström *et al.* 2005). On the other hand, even though *Fsp* is often found in high intertidal pools of varying salinities, it has not colonized the Baltic Sea and no populations are known to have evolved for a permanent low salinity habitat. Ongoing genomic studies will allow us to further explore the molecular mechanisms leading to the convergent adaptations of the hybrids and the polyploid *Fv*.

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